

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| | | |
|--|---|--|
| Applicant's or agent's file reference 341.012W01 | FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. | |
| International application No. PCT/US 99/ 30925 | International filing date (day/month/year) 22/12/1999 | (Earliest) Priority Date (day/month/year) 15/09/1999 |
| Applicant PROMEGA CORPORATION | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 9 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

**THERMOSTABLE LUCIFERASES FROM PHOTURIS PENNSYLVANICA AND PYROPHORUS
PLAGIOPHTHALAMUS AND METHODS OF PRODUCTION**

5. With regard to the **abstract**, ✓

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

61

☒ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/30925

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 76 (11-partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

In claim 11, SEQ ID Nos. 12 and 13 do not encode any amino acid sequence of claim 9 and 10.
Claim 76 refers to itself.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: (1-10,12-46,65-68,73)-complete, (11,58)-partially

abc

1.1. Claims: (1-10,12-46,65-68,73)-complete, (11,58)-partially

abc

1.2. Claims: (1-10,12-46,65-68,73)-complete, (11,58)-partially

abc

1.3. Claims: (1-10,12-46,65-68,73)-complete, (11,58)-partially

abc

1.4. Claims: (1-10,12-46,65-68,73)-complete, (11,58)-partially

abc

1.5. Claims: (1-10,12-46,65-68,73)-complete, (11,58)-partially

abc

1.6. Claims: (1-10,12-46,65-68,73)-complete, (11,58)-partially

abc

2. Claims: 47-57,75

123

3. Claims: 58-partially, (59-64,69-72,74)-complete

Please note that all inventions mentioned under item 1, although not necessarily linked by a common inventive concept, could be searched

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

without effort justifying an additional fee.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 76 (11-partially)

In claim 11, SEQ ID Nos. 12 and 13 do not encode any amino acid sequence of claim 9 and 10.

Claim 76 refers to itself.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/53 C12N15/10 C12N9/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CAB Data, STRAND, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|---|
| X | <p>WO 99 14336 A (HALL MARY P ;PROMEGA CORP (US); WOOD KEITH V (US)) 25 March 1999 (1999-03-25)</p> <p>Fig.46,47 page 34, line 1 -page 42, line 6; table 6 page 47, line 3 -page 61, line 2</p> <p style="text-align: center;">--- -/--</p> | <p>1-6,8, 12,14, 16,17, 19,21, 23,24, 28-37, 47-58, 65,66, 68,73,75</p> |



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

7 August 2000

Date of mailing of the international search report

14.08.00

Name and mailing address of the ISA

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Authorized officer

Hornig, H

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|--|
| X | EP 0 524 448 A (KIKKOMAN CORP) 27 January 1993 (1993-01-27) the whole document ---- | 1-5, 8, 12, 14, 16, 17, 19, 21, 23, 24, 28-37, 58, 65, 66, 73 |
| X | WO 95 25798 A (SECR DEFENCE BRIT ;LOWE CHRISTOPHER ROBIN (GB); WHITE PETER JOHN () 28 September 1995 (1995-09-28) the whole document ---- | 1-5, 8, 12, 14, 16, 17, 19, 21, 23, 24, 28-37, 58, 65, 66, 73 |
| X | WHITE ET AL: "Improved thermostability of the north american firefly luciferase: saturation mutagenesis at position 354" BIOCHEMICAL JOURNAL, GB, PORTLAND PRESS, LONDON, vol. 319, no. 319, 1996, pages 343-350-350, XP002097112 ISSN: 0264-6021 the whole document ---- | 1-5, 8, 12, 14, 16, 17, 19, 21, 23, 24, 28-37, 58, 65, 66, 73 |
| X | WO 98 46729 A (MURRAY JAMES AUGUSTUS HENRY ;SECR DEFENCE (GB); LOWE CHRISTOPHER R) 22 October 1998 (1998-10-22) the whole document ---- | 1-5, 8, 12, 14, 16, 17, 19, 21, 23, 24, 28-37, 58, 65, 66, 73 |
| A | K.V. WOOD ET AL.: "Bioluminescent click beetles revisited" J. BIOLUMINESCENCE AND CHEMILUMINESCENCE, vol. 4, 1989, pages 31-39, XP000906944 JOHN WILEY & SONS, LTD, NEW YORK, US cited in the application the whole document ---- | |
| A | WO 95 18853 A (PROMEGA CORP) 13 July 1995 (1995-07-13) the whole document ---- | |

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|----------------------------|
| A | K.V. WOOD ET AL.: "Complementary DNA coding click beetle luciferases can elicit bioluminescence of different colors" SCIENCE, vol. 244, 12 May 1989 (1989-05-12), pages 700-702, XP002137278 AAAS, WASHINGTON, DC, US cited in the application the whole document ---- | |
| A | K.V. WOOD ET AL.: "Introduction to beetle luciferases and their applications" J. BIOLUMINESCENCE AND CHEMILUMINESCENCE, vol. 4, no. 1, July 1989 (1989-07), pages 289-301, XP000906968 JOHN WILEY & SONS, LTD, NEW YORK, US cited in the application the whole document ---- | |
| A | DEMENTIEVA E I ET AL: "PHYSICOCHEMICAL PROPERTIES OF RECOMBINANT LUCIOLA MINGRELICA LUCIFERASE AND ITS MUTANT FORMS" BIOCHEMISTRY, US, AMERICAN CHEMICAL SOCIETY. EASTON, PA, vol. 1, no. 61, 1 January 1996 (1996-01-01), pages 115-119-119, XP002078631 ISSN: 0006-2960 the whole document ---- | |
| A | L. YE ET AL.: "Cloning and sequencing of a cDNA for firefly luciferase from Photuris pennsylvanica" BIOCHIMICA BIOPHYSICA ACTA, vol. 1339, 1997, pages 39-52, XP000909154 ELSEVIER SCIENCE, AMSTERDAM, NL cited in the application the whole document ---- | |
| X | US 5 605 793 A (STEMMER WILLEM P C) 25 February 1997 (1997-02-25) cited in the application column 16, line 1 - line 6 column 16, line 50 - line 55 ---- | 47-60, 70-72, 74, 75 |
| X | WO 98 13487 A (MAXYGEN INC) 2 April 1998 (1998-04-02) page 39, line 30 - line 31; claims 1-57; figures 1, 3 ---- | 58-60, 70-72, 74 |
| | ----- -/-- | |

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| X | ZHANG J-H ET AL: "Directed evolution of a fucosidase from galactosidase by DNA shuffling and screening" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 94, 1 April 1997 (1997-04-01), pages 4504-4509, XP002087464 ISSN: 0027-8424 the whole document | 47-57,75 |
| X | STEMMER W: "DNA shuffling by random fragmentatio and reassembly: In vitro recombination for molecular evolution" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 91, 1 October 1994 (1994-10-01), pages 10747-10751, XP002087463 ISSN: 0027-8424 the whole document | 47-57,75 |
| A | CADWELL R AND JOYCE G ET AL: "Randomization of genes by PCR mutagenesis" PCR METHODS AND APPLICATIONS,US,COLD SPRING HARBOR, NY, vol. 2, 1992, pages 28-33, XP002087462 ISSN: 1054-9803 the whole document | 47-57,75 |
| A | FROMANT M ET AL: "DIRECT RANDOM MUTAGENESIS OF GENE-SIZED DNA FRAGMENTS USING POLYMERASE CHAIN REACTION" ANALYTICAL BIOCHEMISTRY,US,ACADEMIC PRESS, SAN DIEGO, CA, vol. 224, no. 1, 1995, pages 347-353, XP000486749 ISSN: 0003-2697 cited in the application the whole document | 47-57,75 |

| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
|---|---|---------------------|--|--|
| WO 9914336 | A | 25-03-1999 | AU 9492198 A EP 1015601 A | 05-04-1999 05-07-2000 |
| EP 0524448 | A | 27-01-1993 | DE 69229376 D DE 69229376 T US 5229285 A JP 5244942 A | 15-07-1999 15-06-2000 20-07-1993 24-09-1993 |
| WO 9525798 | A | 28-09-1995 | AU 697883 B AU 1954595 A BR 9507140 A CA 2186144 A CN 1149318 A EP 0751996 A FI 963741 A GB 2301592 A, B JP 9510610 T NO 963983 A | 22-10-1998 09-10-1995 30-09-1997 28-09-1995 07-05-1997 08-01-1997 20-11-1996 11-12-1996 28-10-1997 23-09-1996 |
| WO 9846729 | A | 22-10-1998 | AU 6929398 A EP 0973874 A | 11-11-1998 26-01-2000 |
| WO 9518853 | A | 13-07-1995 | AU 698424 B AU 1830395 A CA 2157476 A EP 0689587 A JP 8510387 T | 29-10-1998 01-08-1995 13-07-1995 03-01-1996 05-11-1996 |
| US 5605793 | A | 25-02-1997 | AU 703264 B AU 2971495 A CA 2182393 A CN 1145641 A EP 0752008 A EP 0934999 A JP 10500561 T WO 9522625 A US 5811238 A US 5837458 A | 25-03-1999 04-09-1995 24-08-1995 19-03-1997 08-01-1997 11-08-1999 20-01-1998 24-08-1995 22-09-1998 17-11-1998 |
| WO 9813487 | A | 02-04-1998 | AU 4503797 A AU 4597197 A EP 0964922 A EP 0963434 A WO 9813485 A | 17-04-1998 17-04-1998 22-12-1999 15-12-1999 02-04-1998 |